EFFECT OF CHEESE WHEY PROTEIN CONCENTRATES ON THE BAKING QUALITY AND RHEOLOGICAL CHARACTERISTICS OF SPONGE DOUGHS MADE FROM HARD RED SPRING WHEAT FLOUR

The U.S. cheese industry produces about 22 billion lb. of fluid whey annually. Approximately one-third is used for food or animal feed, and the rest is essentially wasted (1). Disposal of this material without undue pollution of the environment presents many problems.

One manner of utilizing whey is to process it to enrich its components which may be utilized in more specific applications than that of the whey itself. Small processors may have difficulty economically processing whey using evaporators or specialized equipment. However, the trend is towards centralization of facilities where costs for processing equipment can be covered by the large volume they handle.

In the manufacture of American cheese, after separation of the milk curd, the resulting whey is a watery solution containing 0.9% protein, 0.6 to 0.8% ash, and 4.5 to 5% lactose. Lactose may be used in infant formula, as a pharmaceutical diluent, and in fortifying low-fat milk beverages. Whey protein, denatured or undenatured, is nutritious; its protein efficiency ratio exceeds that of the casein of nonfat dry milk (NFDM) (2). Undenatured soluble whey proteins may find application in whipping, fortifying both soft drinks (3) and nutritional milk-type beverages.

Denatured whey proteins are reported to be suitable for use in baking. Larsen et al. (4) reported that milk serum proteins (whey), when properly heated, have no adverse effect on bread loaf volume. Guy et al. (5) reported that lactose in whey decreases loaf volume. Thus processes which increase protein content and decrease lactose should upgrade whey for breadmaking.

This paper reports on the sponge bread-baking quality and farinograph characteristics of hard spring wheat flour doughs containing whey protein concentrates prepared by both commercial and laboratory sources. Concentrates were prepared utilizing ultrafiltration, gel permeation, and polyphosphate precipitation techniques. It also attempts to explain what characteristics of these protein concentrates can be correlated with baking quality.

Generally preparative procedures for whey protein concentrates aim at producing as soluble a protein preparation as possible. However, Morr et al. (6) report that significant amounts of protein become denatured in preparative procedures involving ultrafiltration, gel permeation, and metaphosphate precipitation even under the mildest conditions. The isolates used in this study

[1]Agricultural Research Service, U.S. Department of Agriculture. Reference to brand or firm names does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

[2]Work conducted at Washington, D.C.

were not further modified by heat to produce additional denaturation.

Whey Protein Concentrates

Only limited information was available on concentrates obtained from commercial sources.

Commercial concentrates 30 and 35 were prepared from electrodialyzed sweet wheys. Commercial concentrate 75 was prepared from gel-permeated mixed acid and sweet wheys. Laboratory-prepared concentrates were prepared in the Dairy Products Laboratory (DPL). All concentrates were in powder form.

Polyphosphate-Precipitated and Gel-Permeated (Poly G)

Commercial cottage cheese whey of pH 4.5 was obtained from a local dairy and collected in bulk tanks over a 3-day period before processing. After settling, the whey was clarified, placed in a 500-gal. cheese vat, and sodium hexametaphosphate dissolved in 82° C. water was added to the whey to a final concentration of 0.5% by weight.

Reagent-grade hydrochloric acid diluted approximately 3:1 with water was next added to the whey mixture to lower the pH to 2.5 and precipitate the whey proteins. The mixture was stirred vigorously for 30 min. and then allowed to settle overnight. As much of the clear supernatant as possible was siphoned off and the remainder, containing the precipitated whey proteins, was then continuously centrifuged in a deLaval (BRP × 207) desludging centrifuge. The precipitate (14 to 17% TS) was resuspended in a small quantity of water and solubilized by adding sodium hydroxide to a pH of 6 to 6.7.

The neutral material was then run through a Sephamatic Gel Filter GF 04-10 packed with Sephadex G-25 Coarse. A 24-liter charge of the concentrate containing approximately 7% protein was applied to the column and eluted at a rate of 4 liters per min. with deionized water supplied by a Culligan demineralization system. The conductivity of the effluent was continuously monitored during elution by a Beckman[3] flow-through conductivity cell and an Industrial Instruments, Incorporated conductivity bridge Model RC 16B2. The high-molecular-weight fractions were collected from each cycle, pooled, pasteurized at 74°C. for 15 sec., and evaporated to 25 to 35% TS in a Wiegand falling film evaporator using a maximum temperature of 63°C. The concentrate was then spray-dried at a 143°C. inlet temperature using a 0.75 mm. nozzle at 126.6 kg.cm.^{2} in a Gray-Jensen spray dryer[3].

Ultrafiltrated and Gel-Permeated (UFG)

Ultrafiltration of cottage cheese whey was carried out according to the method of McDonough et al. (7). The gel permeation was carried out identical to the method described above under Poly G. In the case of UFG 70, the cottage cheese whey was not neutralized with NaOH.

Emulsifier

Sodium stearoyl-2 lactylate (Emplex as a registered name of C. J. Patterson and Company) was used because of its reputed ability to increase the volume of breads fortified with high-protein soy flour or skim-milk solids.

[3]Holsinger, V.H., personal communications (1973).

TABLE I. SPONGE DOUGH FORMULA

Ingredient[a]	Sponge %	Dough %
Flour	70	30
Water	44	Variable
Sugar	...	7.5
Shortening	...	3.0
Compressed yeast	2.3	...
Salt	...	2.25
Whey protein concentrates	...	Variable
Malt	0.5	...
Arkady	0.5	...
Dough temperature	77 ± 1° F.	80-81° F.
Fermentation	4 hr. at 86° F. 90% r.h.	40 min. floor time, 86° F., 15 min. intermediate proof; proof 100° F., 90% r.h., 60 min.
Mix time	1 min. low speed 2 min. second speed	1 min. low speed, variable second speed
Bake		415° F., 25 min.

[a]All ingredients on a flour basis.

Materials

Flour. A commercial lot of bleached, bromated, and malted hard red spring (HRS) wheat flour containing 14.6% protein and 13.7% moisture was used. The flour was kept in sealed bags at −18°C. until used. HRS flour was selected because of its high protein content which is of interest in producing a high-protein-fortified bread product.

Methods

Water Absorption. A Model PL-2H farinograph with the bowl temperature set at 30°C. (86°F.) was used to determine rheological properties of the dough. The mixer rotated at 63 r.p.m. Using the 300-g. constant-flour-weight procedure (8), sufficient water was added to a blend of flour, whey protein concentrate, and 2% salt to center the curve on the 500-B.U. line. Salt was added because it exerted a significant effect on the rheological properties and also is used in the bake formula. Absorptions were corrected to 480-g. dough weight by the methods of Stamberg and Merritt (9) in which plasticity increased 20 farinograph units (equivalent to 0.7% absorption) per 20-g. increase in the weight of the dough.

Baking. The AACC standard method (8) for sponge and dough was followed with modifications as shown in Table I. To obtain a finer grain, the dough was passed through the sheeter roll the second time at 1/8-in. clearance rather than the recommended 3/16-in. Farinograph absorptions of flour with 2% salt and suitable protein concentrate were used to determine the amount of water to use for the bread formula.

Bread Scoring. Breads were scored 10 points each for symmetry, crust color, and crumb color, and 20 points each for texture and grain cell structure. Mounted standards of bread were prepared and assigned numbers to help judge grain and crust color. Volume scores were

TABLE II. ANALYSIS OF WHEY PROTEIN CONCENTRATES

Sample	Protein %	Lactose %	Ash %	pH 7% Solids Solution
UFG 83	83.3	5.5	5.2	6.9
Poly G 78 I	78.7	2.9	13.7	6.7
Poly G 78 II	78.0	1.0	12.8	6.0
UFG 70	69.2	18.9	2.2	4.5
NFDM	36.2	50.6	8.0	6.6
Comcon 35	35.0	55.6	3.0	6.4
Comcon 75	72.8	7.2	13.8	7.3
Comcon 30	30.5	52.4	8.5	6.1

TABLE IV. EFFECT OF PROTEIN SUPPLEMENTS ON TASTE, TEXTURE OF SPONGE BREAD 9-POINT HEDONIC SCALE

Age of Bread	Sample	Taste	Texture
1 Day	Control[a]	7.05	6.70
	2.5% UFG 83	6.95	6.75
	Control[a]	6.95	6.41
	2.7% Poly G 78	6.86	6.95
2 Days	Control[a]	6.95	6.47
	2.5% UFG 83	6.89	6.47
	2.9% Comcon 75	6.74	6.26

[a]Bread with no added protein supplement.

TABLE III. CHARACTERISTICS OF WHEY PROTEIN CONCENTRATES ASSOCIATED WITH THEIR BAKING QUALITY IN HRS DOUGHS AT A 2% PROTEIN/FLOUR LEVEL

Supplements	% Protein Denaturation pH 5.1	% Lactose[a] Added	Farinograph: Minutes stability	Farinograph: % Absorption	% Change[b]: Loaf volume	% Change[b]: Bread scores
2.6% UFG 83	62	0.15	38	64.0	0.0	−0.8
2.7% Poly G 78 (I)	38	0.075	21	62.0	0.0	+1.0
2.6% Poly G 78 (II)	30	0.027	23	60.7	−1.5	−1.4
2.9% UFG 70	13.5	0.55	22	59.8	−6.9*	0.0
6% Comcon 35	41	3.3	31	62.5	−10.2**	−3.1**
2.8% Comcon 75	50	0.2	10	65.2	−10.8**	−1.2
6.8% Comcon 30	11	3.8	25	60.1	−15.2**	−0.8

[a]Basis flour.
[b]Percent change compared to bread with no protein supplement.

not added to loaf quality scores. Volume was expressed in cc. per g. bread cooled 10 min. out of the oven.

Compressibility. A Baker compressimeter was used to test the bread firmness. Loaves were cooled 1 hr. out of the oven, sealed in polyethylene bags, and stored at room temperature. Immediately before testing, bread was uniformly sliced in a machine slicer. Six center slices were tested per loaf. Data were expressed as g. load on the plunger necessary to depress the center of a slice of bread 3 mm. at the No. 1 setting of the instrument. Thus, the greater the load required, the firmer the slice.

Organoleptic Evaluation. Bread slices with crust cut off were judged by 16 to 20 members of the Dairy Products Laboratory for taste and texture. The bread was stored in plastic bags 1 to 3 days at room temperature before evaluation, then sliced. The samples were randomly presented in the DPL panel room using standard lighting. Both taste and texture were evaluated using the 9-point hedonic scale (10). The data were treated statistically (11).

Chemical Assay. The index of protein denaturation in whey protein concentrates was determined using the Rowland procedure (12). It is a measure of the protein insoluble at pH 5.1. The pH 5.1 was selected because it is the isoelectric point of β-lactoglobulin which makes up about 80% of the whey proteins. This also is in the pH range of the sponge and dough at the time of mixing. Morr et al. (6) reported that the pH 4.6 solubility value cannot be used as an index of whey protein in metaphosphate precipitated proteins as its apparent denaturation is too high. In the procedure, a) total nitrogen in a 0.7% solids suspension of the concentrate was determined by the micro-Kjeldahl procedure; b) the clear supernatant after centrifuging this suspension at pH 5.1 was analyzed for total nitrogen; and c) nonprotein nitrogen was determined as 1.4% solids that are soluble in 12% trichloroacetic acid.

$$\% \text{ denatured protein} = \frac{a-b}{a-c} \times 100$$

Lactose was determined by the procedure of Fox et al. (13). Ash was determined by incinerating the samples overnight at 600° C. Moisture was determined by drying powders for 16 hr. at 70° C. Carbon dioxide released from fermenting doughs was determined by using 16.60 g. of the mixed sponge and dough prepared for bread-baking and placing it inside pressuremeter cups equipped with a Hg manometer. The sealed cups were held 5 min. in an 86° F. water bath, degassed, and readings were made after 2 hr.

Statistics

In factorially designed studies the data were treated statistically (11) (see description of samples under *Baking* section of **Experimental Results**—2% protein added). Significant differences (SD) for the values of other bakes were determined by the use of the formula

$$\frac{2S\sqrt{a}}{\sqrt{n}}$$

where S = standard deviation
a = numerical rank apart in the array of data
n = number of replications

Experimental Results

Table II gives an analysis on a dry-weight basis of the concentrates studied. The samples are described by code and numbers, the numbers referring to the approximate protein percent. UFG refers to DPL concentrates processed by ultrafiltration and gel permeation, Poly G refers to DPL concentrates processed by polyphosphate precipitation and gel permeation. Comcon refers to commercially prepared concentrates. NFDM, which is standard in many bake formulas, is also included.

Baking—2% Protein Addition. Table III summarizes the baking quality, farinograph data, and some analytical characteristics of whey protein concentrates. The first two samples and Comcon 75 were factorially evaluated (11) in breads using two absorptions and three mixing times for their doughs. The bake data represent averages of all test conditions. The rest of the samples were evaluated using optimum mixing times and absorptions, as determined by the baker, for their doughs. Twenty-five parts per million of potassium bromate oxidizing agent was used for all samples. Addition of UFG 83 or Poly G 78 had no significant adverse effect on volume or total scores of bread. Relatively sizeable portions of their proteins were denatured; they contained negligible amounts of lactose and produced moderately high or high farinograph stability values. As subjectively determined by the baker, UFG 83 increased dough-water absorptions by 4%. Mixing requirements of its doughs were not changed. Volume and bread scores were not changed by Poly G 78 but dough mixing times were decreased by 1 min. and clean-up times slightly extended. Comcon 75 in which 50% of the protein was denatured contained only 0.2% lactose, but it significantly lowered specific bread volume. However, it produced a low farinograph stability time of only 10 min.

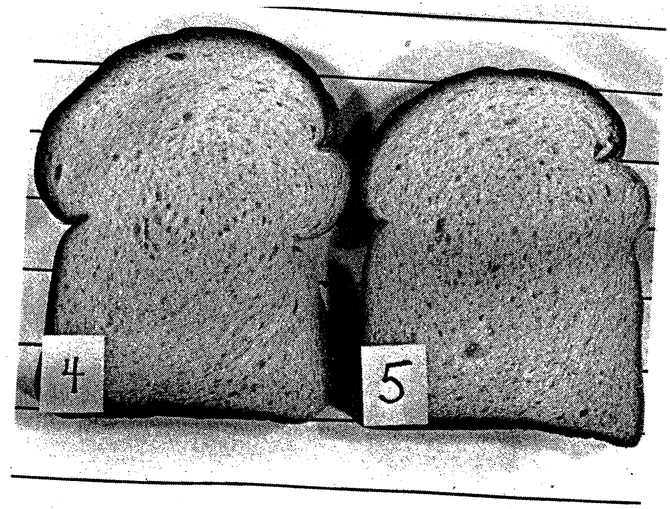
Supplements UFG 70, Comcon 30, and 35 significantly decreased bread volume (Table III). They contained high levels of undenatured protein, or lactose or both to account for this decrease.

Figure 1 shows that the center slice of bread (No. 5) supplemented with Comcon 75 is smaller than slice (No. 4) from the water control bread. Slice of bread (No. 6) supplemented with UFG 83 is slightly larger than slice (No. 4) from the water control bread.

Correlation of Farinograph Absorption and Protein Denaturation. Farinograph absorption of doughs containing 2% protein concentrates plus 2% salt was significantly correlated ($r = 0.915$) with percent protein denaturation in the protein concentrates (Fig. 2), indicating that protein denaturation increased water absorption.

Keeping Quality. Compression of slices held up to 6 days at room temperature showed that addition to doughs of 2.5% UFG 83 yielded, slightly firmer bread, initially and in storage; and 2.9% Comcon 75 yielded still firmer bread than that of the control (Fig. 3). Supplementation with 2.6% Poly G 78 slightly increased, initially and in storage, the firmness of bread while 6% NFDM increased tenderness (Fig. 4).

Panel Scores. In spite of the fact that 2% protein supplements increased firmness as shown by the compressimeter, taste and texture scores of breads were not changed significantly with them (Table IV).



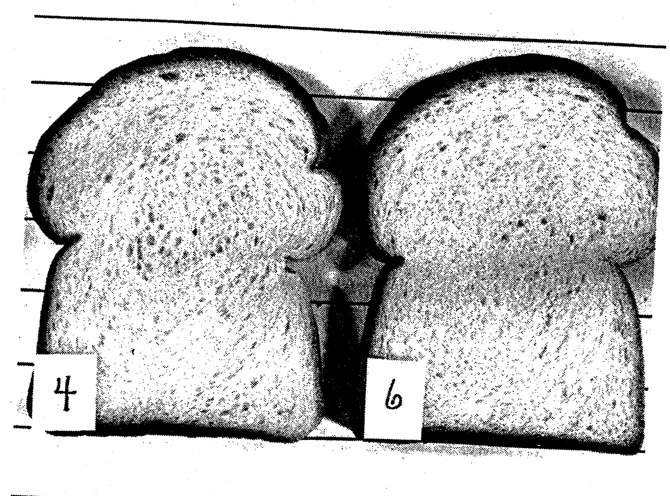


Fig. 1. Effect of protein concentrates on bread at a 2% protein level. No. 4 = water control; No. 5 = 2.9% Comcon 75; No. 6 = 2.6% UFG 83.

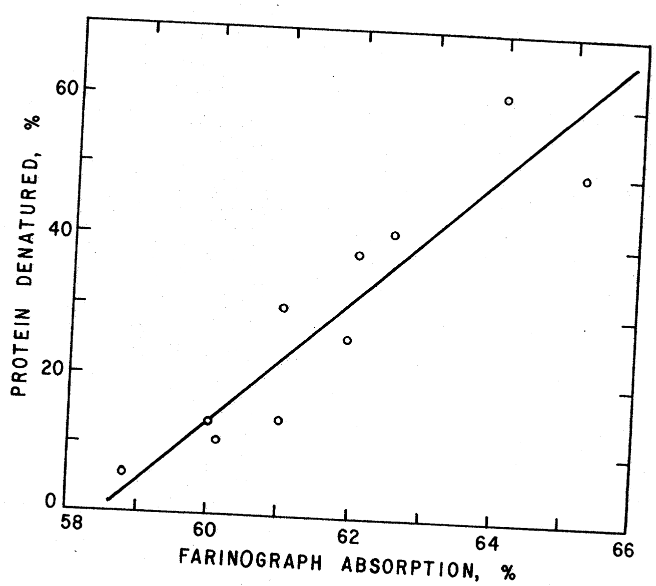


Fig. 2. Correlation of percent farinograph absorption of doughs containing 2% protein concentrates and 2% salt vs. percent denatured protein in the concentrate.

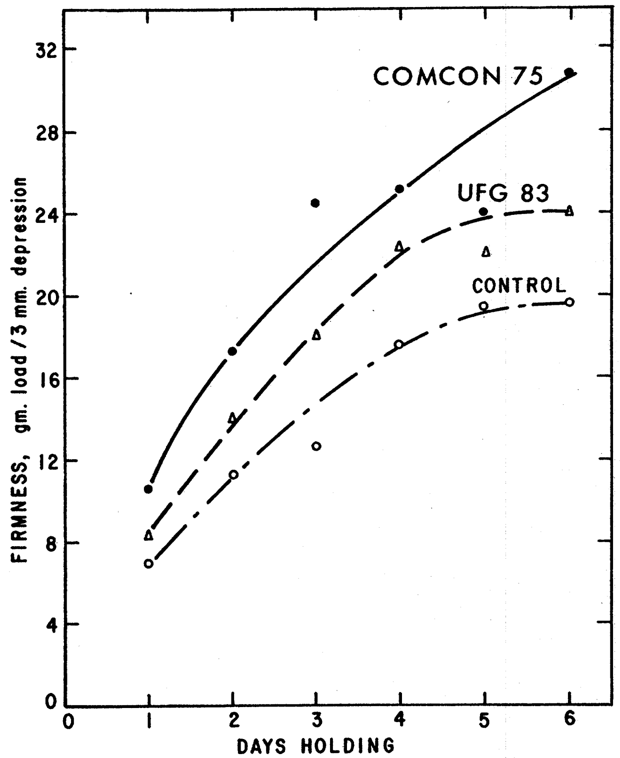


Fig. 3. Effect of protein concentrates at a 2% protein level on bread staling.

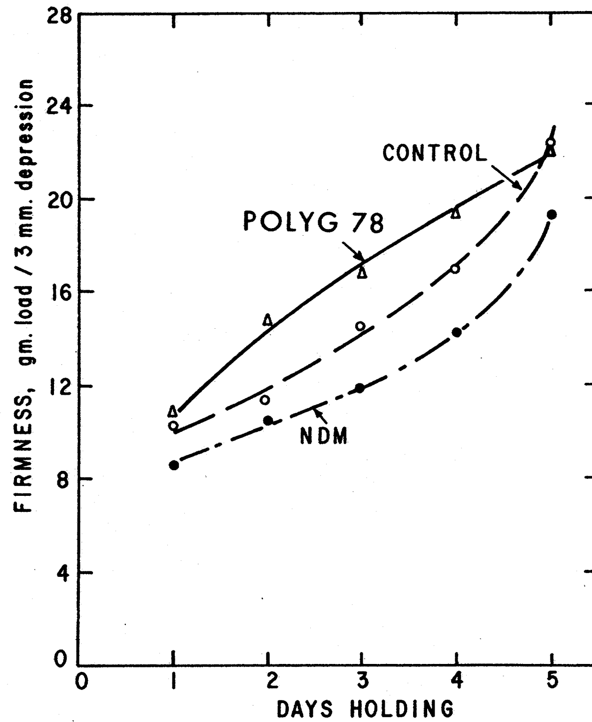


Fig. 4. Effect of Poly G 78 and NDM at a 2% protein level on bread staling.

Effect of Increased Protein Levels

Baking. Although not significant in all cases, Table V shows that the addition of 0.5% Emplex emulsifier (sodium stearoyl-2-lactylate) increased loaf volume, bread scores, and CO_2 production of doughs containing 4% added protein from UFG 83 and Poly G 78. Emplex significantly increased the specific volume and CO_2 production of doughs with 6% added protein but not the volume of the water control. Emplex, when used with 4% added protein increased bread volumes equivalent to the water control. When used with 6% added protein it increased bread volumes equivalent to a 3% NFDM control. Addition of Poly G 78 decreased symmetry scores and caused capping, but produced excellent crumb color and grain. Average loaf volumes were computed from 4 to 8 loaves and CO_2 pressures from 2 to 3 replications. Optimum water absorptions and mixing times were used for each of the protein concentrates.

Keeping Quality. Addition of whey protein concentrates toughened bread as measured by the compressimeter.

With 4 or 6% added protein, addition of 0.5% Emplex to UFG 83-containing breads increased softness initially and through 4 days of storage (Table VI). These slices were softer than the controls with no Emplex. With Emplex, the bread slices containing 4% Poly G 78 had about the same softness as the water-bread control slices. With 6% added protein from Poly G 78, slices of this

TABLE V. EFFECT OF EMPLEX ON THE SPECIFIC VOLUME, BREAD-BAKING QUALITY AND CO_2 PRODUCTIONS OF DOUGHS WITH ADDED PROTEIN CONCENTRATES

		% Water Absorption of the Dough	% Emplex: Specific vol. cc./g. 0		Specific vol. cc./g. 0.5	Bread score 0	Bread score 0.5			
4%[a] Added protein	Water control	60	6.37		6.34	59.3	59.6	188	*	211
	UFG 83	66	6.03		6.36	58.3	59.3	196		204
	Poly G 78	61	5.91	*	6.31	57.8	59.7	183		185
6%[a] Added protein	3% NFDM	62	6.77		...	61.7	...	197		...
	UFG 83	66	6.10	*	6.60	61.3	61.8	197	*	211
	Poly G 78	61	6.12	*	6.70	60.1	60.3	193	*	208

[a]40 p.p.m. potassium bromate added to all doughs.

TABLE VI. EFFECT OF PROTEIN CONCENTRATES ON BREAD KEEPING QUALITY

4% Protein Added
g. to Depress Slice 3 mm.

	Water control	UFG 83 +0.5% Emplex	Poly G 78 +0.5% Emplex
Days 1	10.0	6.7	12.4
2	13.0	8.0	11.5
3	14.3	10.7	15.3
4	16.5	11.3	16.7

g. 6% Protein Added

	3% NFDM Control		UFG 83		Poly G 78	
Emplex	0	0.5%	0	0.5%	0	0.5%
Days 1	9.5	7.5	10.3	8.5	17.3	12.3
2	13.5	7.2	17.2	10.3	22.3	14.7
3	16.7	10.7	19.2	12.3	22.0	18.7
4	18.0	13.2	21.8	15.0	...	23.8

TABLE VII. AVERAGE PANEL SCORES OF BREADS CONTAINING WHEY PROTEIN CONCENTRATES WITH 0.5% EMPLEX

Age	Water Control	Control 5% NFDM	4%[a] UFG 83	4%[a] Poly G 78	6%[a] UFG 83	6%[a] Poly G 78
			Average taste			
1 day	6.62	7.06	...	...	6.89	6.62
3 days	7.12	6.82	...	...	6.28	6.00[b]
1 day	6.91	7.26	6.50	6.66	...	...
3 days	6.88	6.50	6.66	6.76	...	...
			Average texture			
1 day	6.35	6.45	...	...	6.63	6.35
3 days	6.94	6.71	...	...	6.36 (soft)	6.36 (sl. firm)
1 day	6.67	6.72	7.00	7.00		
3 days	6.11	6.76	6.00 (soft)	6.83		

[a]On a protein added level.
[b]Significantly lower than the water control.

bread were slightly firmer than the 3% NFDM-containing slices with no added Emplex.

Panel Scores. Breads with either 4 or 6% level of protein had satisfactory taste and texture (Table VII). On the third day, however, taste scores of bread slices containing 6% protein levels were somewhat lowered. When the bread slices were either softer (UFG 83) or firmer (6% Poly G 78) than the controls (with no added Emplex), as measured by the compressimeter, texture scores on the third day were slightly lowered.

Conclusions

With the exception of Comcon 75, 2% whey protein concentrates could be used in bread without loss of baking quality if they contained high levels of protein, if the protein was denatured substantially or in part, and if the lactose content was low. Also their doughs showed high or relatively high farinograph stability times.

Comcon 75 met all of the above mentioned criteria except it showed a low farinograph stability value. It also performed poorly in the bake test. Thus all criteria should be met for satisfactory performance.

Water absorption of whey protein concentrate-containing doughs, as measured by the farinograph method, correlated directly with the percent of the whey protein that was denatured (Fig. 2).

Relatively high levels of whey protein (4 to 6% of flour weight) could be used in sponge bread-baking without loss in loaf volume or quality score in the finished product if 0.5% sodium stearoyl-2-lactylate (Emplex) was included in the dough formula. This held only for the inclusion of whey protein concentrates that demonstrated, when used at lower concentrations, good functionality for baking.

Poly G whey protein concentrates displayed unique behavior when used in baking. When Poly G proteins were used at 4 or 6% with added Emplex, the bread had improved grain and crumb color compared to water or NFDM control loaves but poorer break in shred. The factor or factors responsible for the poor break and shred are unknown. Nevertheless, these data indicated that the methods used to prepare whey protein concentrates markedly affected the manner in which doughs containing them handled and baked.

Acknowledgments

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